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Assessment Serum Levels of Neopterin, IL-6, IL-1β, hs-CRP, TNF-α and MMP 9 in Iraqi Rheumatoid Arthritis Patients

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ABSTRACT

Background
Rheumatoid arthritis (RA) is a sore public prolonged arthropathy of indefinite etiology.
Objective
To explore the sera concentrations of neopterin (NPT), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), high sensitivity C-reactive protein (hs-CRP) and matrix metalloproteinase-9 (MMP-9) in RA patients.
Methods
The work involved 40 RA Iraqi patients and 40 healthy control (HC) matched by age and sex. Sera concentrations of neopterin, TNF- α, IL-1β, IL-6, MMP-9 and hs-CRP were estimated by commercially available ELISA kit.
Results
Sera concentrations of neopterin, IL-1β, IL-6, MMP-9, TNF-α and hs-CRP were importantly greater (P<0.001) in RA cases than HCs. There was an important positive link between TNF-α and IL-1β.
Conclusion
These data establish that RA cases have great concentrations of inflammatory indicators, and these values are linked with the neopterin. Current results submit a potential function of these indicators in RA pathogenesis. Furthermore, these parameters can be served as signs of illness action in the identification and therapy of RA.

Keywords: Neopterin, Interleukin-6, Interleukin-1β, Rheumatoid Arthritis

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INTRODUCTION

RA is distinct of the greatest public autoimmune illnesses described by advanced and continuing irritation of diverse structures, particularly the synovia of the junctions driving to joint injury, reduced value of life, and a smaller existence expectation e [1]. Irritation is the hint contributing factor and main mechanism causing in incapacity and amplified death in RA cases [2].

Therefore, irritation valuation in RA with favorite indicators is significant to distinguish continuing product. The best indicators used for these goals are the erythrocyte sedimentation rate (ESR) and CRP in repetition of circadian [3].

Reviewing parameters in rheumatology extremely seemed from the requisite to recognize the mechanisms essential certain rheumatic illnesses. Find out different biomarkers with significant functions in dissimilar periods of growth is still an issue of importance for RA [4]. A numerous of features must been recommended as bio-indicators of illness action in RA cases containing matrix protein of cartilage oligomeric, interleukins and oxidative stress [5-7].

Neopterin, a pyrazino-pyrimidine compound, is produced via macrophages and monocytes in reply to interferon-γ (IFN- γ) manufactured via stimulated T-cells. It is an indicator of cellular immune reply, and levels are raised in situations of macrophages and T-cell activation, including autoimmune illnesses like RA and systemic lupus erythematosus [8]. NPT is establishing at amplified concentrations in biological liquids from inflammatory illnesses persons. The living function of this pteridine still unclear; nevertheless, because of its ability to rise hemeoxygenase-1 content, it has been suggested as a defensive mediator through tension of cellular [9]. As a result, we calculated the special effects of neopterin on RA patients.

The pro-inflammatory interleukin (IL-) 6 controls development of chronic inflammatory diseases, like RA, inflammatory bowel diseases and asthma, similarly including in cardiovascular disorders including atherosclerosis and in development many types of tumors [10].

The pro-inflammatory interleukin-1 is manufactured via the inflammation synovium and seems to exert a main function in the damaging design related with RA [11]. It is an identified stimulating of collagenase for stromelysin and prevents the production of collagen and proteoglycan; improved concentrations of IL-1β and IL-1α have been estimated in RA patients [12].

IL-1 is predominantly made via, dendritic cells (DCs), monocytes and macrophages, and it prompts many interleukins and cell adhesion molecules [13]. IL-1 similarly including proliferation and lymphocytes stimulation [14], autoimmunity progress via reducing T cells tolerance [15].

Another possible indicator for amplified RA risk maybe hs-CRP, where hs-CRP is an excellent indicator of complete inflammatory and is raised in cases with RA [16]. Inflammatory changes exert an essential function in the pathogenesis of RA. Indicators of inflammatory like, IL-6, TNF-α and hs-CRP are greatly expressed in synovium liquid and serum of RA cases and exert a central function in the RA pathophysiology [17]. As a result, we designed the current paper to study the sera levels of these immune biomarkers and increasing risk of RA incident Iraqi patients.

TNF-α is essential pro-inflammatory cytokine controlled of irritation and joint damage in RA. TNF-α and its receptors are freely identified in serum and synovial liquid of RA cases. Harshness of RA is related with TNF- α level in cases with RA [18].

Matrix metalloproteinases (MMPs) are a set of Zn^{2+} reliant extra cellular enzymes which have a main function in a pathological and usual tissue. The complete collection can be separated into sub classes, like, gelatinases, collagenases membrane type MMPs and stromelysins, MMPs in RA was showed to be including in extreme dilapidation of joint tissue [19]. Previous study recommended that MMP-9 could be including in the-helper kind 2 (Th2) growths of T allergen replies [24].

MATERIAL AND METHODS

The current paper was carried out in Al-Fallujah teaching hospital at Al-Anbar Governorate/ Iraq, from October 2017- April 2018. A total of 40 RA patients (30 female and 10 males aged 42-63 year), the diagnosis of RA was made on the basis of the recommended criteria by WHO [20]. Forty age and sex matched (30 female and 10 male aged 41-64 year) healthy individuals served as controls who attended for routine health check up at the hospital. None of the healthy control was taking any medication or nutritional complement; they were carefully chosen after full physical investigation and laboratory tests.

Samples collection: 5 ml. venous blood specimens were assembled in plain tubes, the samples were permitted to coagulate for one hour following which a specimens were centrifuged for 20 minutes at 4000 rpm. Then serum was stored immediately at -20 °C until use.

Serum concentrations of neopterin, IL-6, IL-1β, hs-CRP, TNF-α and MMP 9 were determined by ELISA using a commercial kit manufactured by Mybiosource Company. Micro ELISA system (washer and reader) (Thermo, Germany) and incubator (Gallenkamp, U.K.) were used in ELISA determination.

STATISTICAL ANALYSIS

All Data are presented as mean ± S.D., standard error of mean was computed and differences between means were assessed by the Student t test. Dissimilarities were assumed important at $P < 0.05$. All statistical analysis was done by SPSS statistical software (version 19). The statistical significance, direction and strength of linear association among two measurable parameters, one of which being a normally distributed variable, was measured, and P-value less than the 0.05 was assessed as a statistically significant difference.

RESULTS

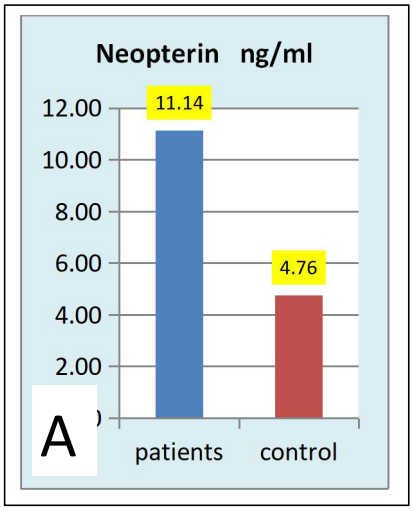
Table no.1shows the average ages of the RA and control subjects were (56.78±5.80) and (55.15±6.70) years, respectively with non-significant differences ($p > 0.05$).

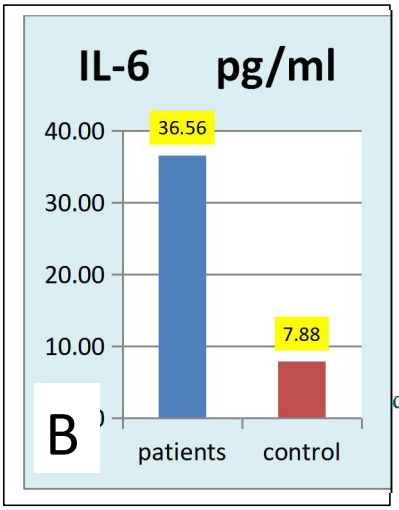
Serum NPT, IL-6, IL-1β, hs-CRP, TNF-α and MMP 9 concentrations were importantly greater ($p<0.001$) in cases RA when compared to control group as exposed in table no.1, (figures 1-A, 1-B, 1-C, 1-D, 1-E and 1-F) respectively.

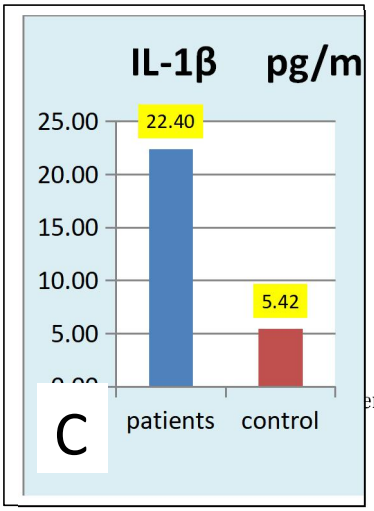
The normal levels of neopterin, IL-6, IL-1β, hs-CRP, TNF-α and MMP9 were (4.76 ± 2.34 ng/mL), (7.88 ± 3.52 pg/mL), (5.42 ± 2.38 pg/mL) (2.30 ± 0.59 ng/mL), (4.19 ± 2.23 pg/mL) and (29.38 ± 20.03.19 ng/mL), raised in cases with RA who recorded (11.14 ± 5.09ng/mL), (36.56 ± 18.58 pg/mL), (22.40 ± 9.85 pg/mL), (6.11 ± 1.74 ng/mlL), (9.91 ± 3.49 pg/mL) and (167.14 ± 65.20 ng/mL), respectively.

Table (1): Assessment of different markers among HC and RA patients

Markers		Mean ± S.D	SEM	T test	p value
Age years	patients	56.78±5.80	1.637	1.074	>0.05
	control	55.15±6.70	1.421		
Neopterin ng/mL	patients	11.14 ± 5.09	0.77	7.253	<0.001
	control	4.76 ± 2.34	0.37		
IL-6 pg/mL	patients	36.56 ± 18.58	2.80	9.603	<0.001
	control	7.88 ± 3.52	0.56		
IL-1β pg/mL	patients	22.40 ± 9.85	1.48	10.623	<0.001
	control	5.42 ± 2.38	0.38		
hs-CRP ng/mL	patients	6.11 ± 1.74	0.26	13.144	<0.001
	control	2.30 ± 0.59	0.09		
TNF-α pg/mL	patients	9.91 ± 3.49	0.53	8.869	<0.001
	control	4.19 ± 2.23	0.35		
MMP-9 ng/mL	patients	167.14 ± 65.20	9.83	12.819	<0.001
	control	29.38 ± 20.03	3.17		







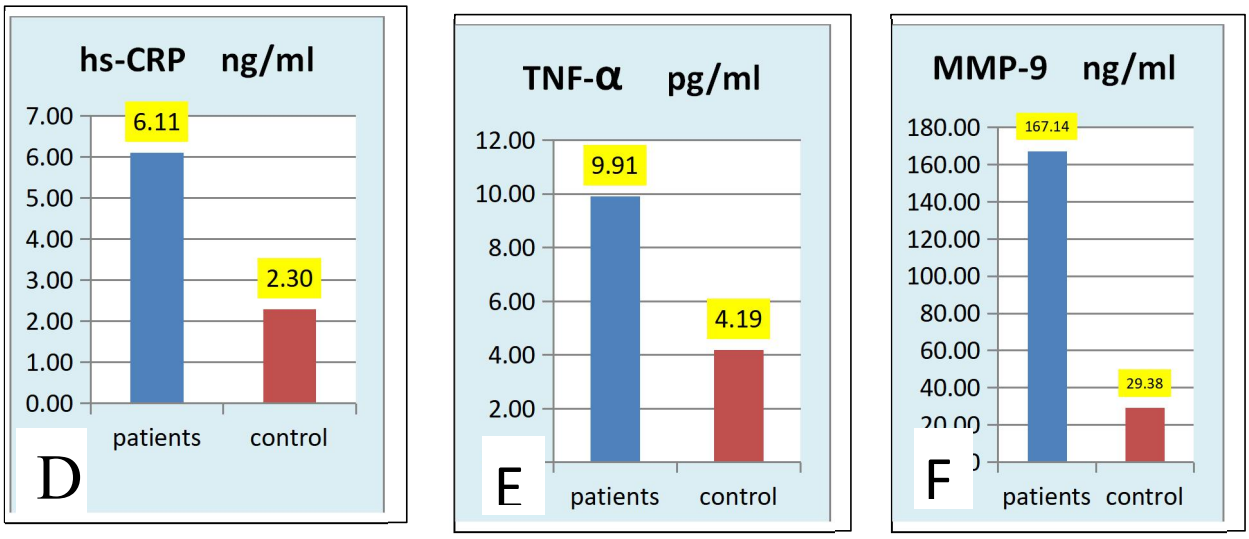


Fig. 1: Represent Man± S.D of Studied Variables in RA Cases Compared to HCs.

The Pearson association test indicated a strong positive association among serum TNF-α with IL-1β as presented in table no.2 (figure 2). In additional, there were important positive associations among serums neopterin with IL-6 hs-CRP, as presented in table no.2, while IL-6 showed positive correlations IL-1β, hs-CRP and MMP-9, also TNF-α showed positive correlations with MMP-9 as presented in table no.2.

Table 2: Pearson Correlation Coefficients of Studied Parameters with Each Other.

Parameter	Neopterin ng/mL	IL-6 pg/mL	IL-1β pg/mL	hs-CRP ng/mL	TNF-α pg/mL	MMP-9 ng/mL
NPT ng/mL	1	.353**	.232	.437**	.244	.256*
IL- pg/mL			.417**	.491**	.299*	.318*
IL-1β pg/mL				.206	.893**	.336*
hs-CRP ng/mL					.204	.445**
TNF-α pg/mL						.329*
MMP-9 ng/mL						1

****. Represent p< 0.01; *. Represent p< 0.05**

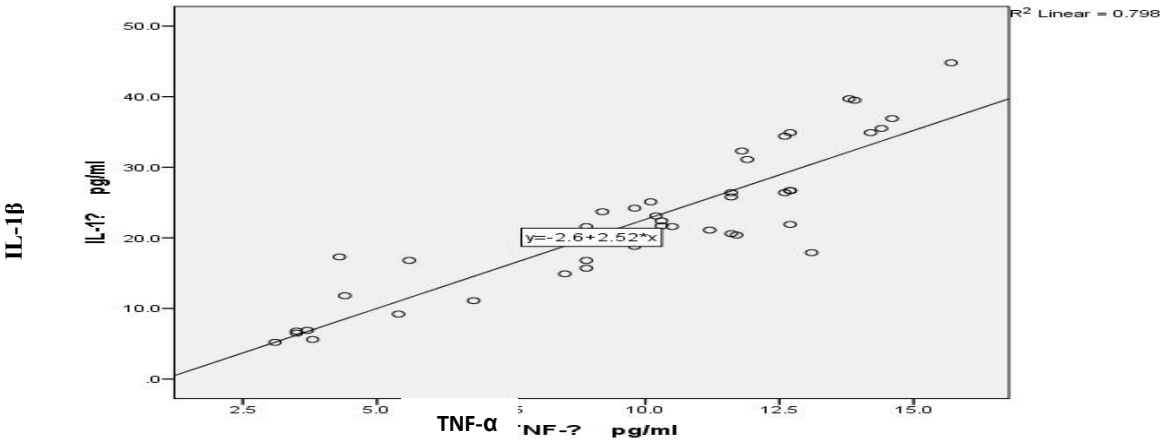


Fig. 2: The relationship among serum IL-1β and TNF-α in Cases with RA.

DISCUSSION

This paper designed to study the effect of RA on NPT, IL-6, IL-1β, hs-CRP, TNF-α and MMP which assessed by ELISA, also to evaluate the variables related to each other and to compare their concentrations in RA compared to HCs. Our outcomes demonstration high elevates of these parameters RA patients by great degree.

The current research showed a great concentration of serum NPT (2.34 times), as an indicator of macrophage activation, in RA cases. NPT is important evidence for infection in RA cases. Proceeding investigates of NPT in RA have been achieved to explain the connection among NPT and illness action. Generally, they establish that NPT linked with illness action in RA.

Previous study showed the high levels of chemokines in the progression of inflammation in RA cases stimulated manufacture of NPT; they establish great NPT concentrations in cases with RA, this consistent with present study [21], as NPT an appropriate biomarker of irritation so elevated concentrations of this biomarker has been reported in the preferentially formed in reply to interferon-γ (IFNγ), and, for the reason that of its help to increase radicals problems in several systems of cellular tissue, NPT is cytokine with pro- inflammatory properties which maybe stimulate macrophage of human [22]. IFNγ stimulated the manufacture of NPT by macrophages which accumulated locally, then release it into SF which work on T lymphocyte system stimulated [23].

Serum levels of IL-6 was importantly lesser in healthy controls (0.215 time) compared RA patients. High IL-6 concentrations were established in serum and synovial liquid in RA cases [24]. These concentrations associated with medical signs like the sum of joints, clinical signs of illness and morning rigidity [25]. Although the accurate reason of RA remnants unidentified, it has been proposed a several steps are necessary to RA progress and development [26]. IL-6 seems to exert an important function in all stages for RA development [27]. It participates in the production of auto antibodies such as anti-citrullinated peptide antibody (ACPA) and rheumatoid factor (RF) and, via plasma blast performing [28]. IL-6 has been confirmed to exert key function in producing of native irritation in joint damage via making endothelial cells to yield monocyte chemoattractant protein-1, IL-8, and to stimulate communication of employment of leukocytes and molecules of adhesion to participate junctions [29].

IL-1, a pro-inflammatory interleukin and a potent transmitter between cells, exerts vital roles in pathogenesis of RA [30]. The pathogenesis of RA remnants to be not completely explained, but a significant story includes association among numeral diverse cells of inflammation, especially T lymphocytes, cells local in the intersection and macrophages. They link by system of proteins identified as interleukins, certain of which play regulatory of immune system or anti-inflammatory cytokines and others which afford pro inflammatory properties. In common physiology, these interleukins are sustained in equilibrium; whatever, in RA the equilibrium moves to the proinflammatory interleukin direction, in RA the best significant pro inflammatory interleukins are IL-1 and TNF-α [31]. These interleukins yield an overlapping properties of biological activities, but it is probable that they performance may show with many interleukins to yield numerous of their pathophysiological functions, TNFα and IL-1 exert a significant function in the linked between the numerous cells in the rheumatoid intersections [32]. They similarly encourage manufacture of chemokines, which offer vital indicators for the cell penetration growth.

TNFα and IL-1β initiate the differentiation of osteoclasts via encouraging T cells to yield receptor activator of nuclear factor kappa-B ligand (RANKL), and they together stimulate complete osteoclasts connecting to damage of sub chondral bone [33]. TNFα encourages IL-1β manufacture and.IL-1β encourages TNFα making. The comparative significance of TNFα and IL-1β in RA greatly discussion. Certain animal models propose that IL-1β exerts an extra vital function in RA progressions including iintersections damage; however TNFα is extra significant in inflammation developments [34]. RA can be discouraged via close the IL-1β receptor or via anti-TNFα. The current paper conducted a powerful positive relationship among IL-1β and TNF-α serum levels as a biomarkers of increasing incident RA.

hs-CRP possibly will be extra strictly related with irritation of tissue in RA diagnostic than the anti-CCP antibody and further auto antibodies preceding RA. It is probable that the incorporation of anti-CCP antibody and hs-CRP concentration close to the period of identification which powerfully guesses occurrence illness. However, outside existence basically a parameter of irritation, hs-CRP could reveal the molecular mechanisms of illness statuses. For instance, hs-CRP manufacture via liver cells, the chief component of this acute-phase interaction, seems to be controlled principally via interleukins 1 and IL-6. Moreover, hs-CRP perhaps need significant immune-modulating purposes as it has been presented to require numerous parts in native immune replies and tissue injury, and has the facility to stimulate the complement system, and increase opsonization phagocytosis [35].

Furthermore, we detected a significant relationship of serum hs-CRP with IL-6 concentrations. Submitted the identified mechanistic connection of IL-6 and hs-CRP, this proposes that IL-6 manufactured in the pretentious joint may in portion be in control for the increasing in hs-CRP realized in these residents of cases. It was suggested that IL-6 manufactured at limited places is quickly from the circulation, and could gather inside the liver to encourage an acute-phase protein response [36, 37].

Even if nearly wholly memberships of the MMP family have been recognized in RA synovium, MMPs-1, 3, 9 and 13 appear to be the greatest significant for joint devastation [38].With further studies on cytokines and MMPs, it has been establish that multiple cytokines and MMPs were involved in cartilage destruction in a complex regulatory networks rather than single factor [39]. MMP-9 is one proteinase de-grading cartilage extracellular matrix (ECM). Under normal conditions, MMP-9 secretion is maintained at homeostasis. The amount of secretion is of critical importance for maintaining integrity of cartilage. MMP-9 level was elevated to altered degrees in RA patient's serum [40]. In collagen-induced inflammation, raised intermediaries of pro-inflammatory like TNF-α which stimulate MMP-9 action in native inflammatory cells, such as synovial fibroblasts of RA, leading to bone damage [41].

The current work has a number of restrictions. It was cross-sectional in strategy, and for those reason fundamental implications it is impossible to be painted. Similarly, the properties of the nature of treatment on NPT concentrations were not surveyed; therefore their consumption as an indicator for treatment effectiveness impossible to be assessed. The comparatively minor model extent may perhaps responsible for inadequate statistical power. Bigger sample sizes would require augmented quantities inside all RA groups, permitting for better accuracy in evaluating the pro-inflammatory indicators among RA patients.

The current paper concluded that high levels of NPT, IL-6, IL-1β, hs-CRP, TNF-α and MMP9 in Iraqi RA was a risk state for RA progress, also TNF-α showed a strong positive correlation with IL-1β in RA patients that means we maybe use these biomarker in diagnosis and treatment of RA disease.

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